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Abstract

The effect of combined iron, silicate, and light co-limitation was investigated in two Southern Ocean diatom species, *Chaetoceros dichaeta* and *Actinocyclus*, sp. and one cosmopolitan species, *Chaetoceros debilis*, all isolated in the Southern Ocean (SO). We found species specific differences in the level of nutrient limitation and its effect on physiological and morphological parameters.

Growth of all species tested was clearly co-limited by iron and silicate, reflected in a 4 to 40 times higher increase in cell numbers in the high iron, high silicate treatments compared with the controls. However, the effect of iron and silicate availability on chain length and frustules structures was species specific. Most drastic frustule malformation was found under iron and silicate co-limitation in *C. dichaeta* while Si limitation caused a strong cell elongation in both *Chaetoceros* species. Additional a significant increase in chain length was observed in these species under high iron conditions. Therefore, species composition in the SO is likely also indirectly affected by these nutrients via different effects on diatom grazing protection. These morphological changes reflect a potential as biological markers in sediments for the growth history of chain forming species.

High light conditions, comparable with light intensities found in the upper 28 m of the SO, showed a negative impact on growth of the endemic species *C. dichaeta* and *Actinocyclus* sp. This is in contrast to the assumed light limitation of SO diatoms and indicates an adaptation strategy to the deep mixing and resulting low light conditions in the SO. In contrast to that, the cosmopolitan species *C. debilis* was not negatively affected by increased light intensity, indicating adaptation to a broader light environment. These results suggest that light limitation of SO phytoplankton due to deep wind mixed layers may play a minor role than hitherto assumed.

1 Introduction

Diatoms are an extraordinary phytoplankton class, which play a major role in global carbon fixation in all regions of the world's ocean (Sarthou et al., 2005). Especially in the SO, diatoms tend to dominate the phytoplankton community and account for as much as ~75% of the annual primary production (Nelson et al., 1995; Tréguer et al., 1995). Diatoms can build up enormous blooms and, since there is only little frustule dissolution during the transport to the deep sea (Tréguer et al., 1989), they are responsible for almost all of the silica sedimentation in the SO (Abelmann and Gersonde, 1991). Besides the macro nutrients nitrate and phosphate that are essential for the growth of all algae, diatoms also depend on the availability of silicic acid ($Si(OH)_4$) to produce their frustules.

While nitrate concentrations are high everywhere in the SO (about 25 μM Dafner and Mordasova, 1994; Tréguer and Jacques, 1992) dissolved Si concentrations vary from 1 to 15 μM north of the Polar Frontal Zone (PFZ) to about 40 to 60 μM on the south side (Coale et al., 2004; Franck et al., 2000; Tréguer and Jacques, 1992). The high Si concentrations south of the PFZ create a favorable environment for diatoms while the low Si concentrations can limit diatom growth (Brzezinski et al., 2005; Coale et al., 2004; Franck et al., 2000; Leblanc et al., 2005).

Besides Si the trace metal iron is known to limit phytoplankton growth in general in the SO. Several in situ iron fertilization experiments in the SO proved that especially the growth of large, chain-forming diatoms was enhanced due to iron addition (see review in de Baar et al., 2005). Nevertheless recent studies showed that diatoms in all size classes were able to benefit from iron fertilization (Hoffmann et al., 2006). Besides the effect on cell growth, iron fertilization increases the maximum specific uptake rates of silicic acid in SO diatoms and enables them to fulfill their silica needs even in water with very low Si concentrations (Brzezinski et al., 2005; De La Rocha et al., 2000; Franck et al., 2003; Franck et al., 2000). It is suggested that this is caused by an increase in the number of active Si transporters in the cell membrane (De La Rocha et al.,

2000). Therefore iron is often described as the proximate limiting factor for community production (Blain et al., 2002; Hutchins et al., 2001; Sedwick et al., 2002) but a co-limitation of iron and silicate is suggested for SO diatoms (Leblanc et al., 2005). It is further suggested that besides growth parameters phytoplankton composition is also affected by iron and silicate and the sensitive interaction of both in the SO (Banse, 1991; Hutchins et al., 2001; Leblanc et al., 2005). Iron requirements of different diatom species seem to be variable and dependent on their photosynthetic architecture as published by Strzepek and Harrison (2004). They describe that open ocean diatoms have developed low iron requirements in general, while coastal species have the ability to adapt to low Fe. This would suggest that diatoms of low Fe regions, such as the SO, can maintain high growth rates under low Fe because they have developed a photosynthetic apparatus that is as effective as others under high Fe.

The extremely deep mixing and the resulting low light intensities are discussed as a third main factor influencing algal growth in the SO (Mitchell et al., 1991; Timmermans et al., 2001; van Oijen et al., 2004). A significant negative correlation of the wind mixed layer (WML) depth and maximum chlorophyll a concentrations (mg m^{-3}) were found in almost all in situ iron fertilization experiments (de Baar et al., 2005). Since light serves as the source of energy for photosynthesis, light intensity and duration determines the degree of photosynthetic activity. The majority of intracellular iron is required in the photosynthetic apparatus and iron limitation lowers the photosynthetic efficiency of phytoplankton (Greene et al., 1994). This suggests that phytoplankton species growing in iron limited regions are suffering more from low light conditions. In other words the cellular iron demand is enhanced under low irradiation (Raven, 1990; Strzepek and Price, 2000). Light limited cells of the diatom *Thalassiosira weissflogii* contained four times more Fe per C compared to controls (Strzepek and Price, 2000). Based on these findings they suggest that photoacclimation of phytoplankton could be affected by the availability of Fe and that Fe limitation could be modulated by light. Since the SO is characterized by low iron and low light conditions most of the year, phytoplankton growth is thought to be co-limited by both factors in this High Nutrient Low Chlorophyll

(HNLC) region (Timmermans et al., 2001). However, laboratory experiments suggest species specific differences in the exact impact of iron and light co-limitation (Sunda and Huntsman, 1997; Timmermans et al., 2001).

Here we present the first study examining the effect of iron, light, and silicate co-limitation on two Antarctic diatom species *Actinocyclus* sp. and *Chaetoceros dichaeta* and one cosmopolitan species *Chaetoceros debilis*, all isolated in the SO, in laboratory experiments. The species are important contributors to the phytoplankton community in the SO and were chosen because of their different size in order to investigate possible size dependent reactions. Further, both *Chaetoceros* species are chain forming and we intended to compare those to a solitary species. We especially turned our attention to the interaction of these three abiotic factors on diatom growth, as well as on physiological conditions and morphologies, and the implications for the SO phytoplankton community structure and paleooceanographic record.

2 Material and methods

The three diatom species *Actinocyclus* sp.,*Chaetoceros dichaeta*, and *Chaetoceros debilis* were isolated on board RV "Polarstern" during the SO iron fertilization experiments EisenEx (*Actinocyclus* sp.) and EIFEX (*Chaetoceros dichaeta*, and *Chaetoceros debilis*). Single cells were isolated under a light microscope using small glass pipettes and rinsed at least three times in sterile filtered Antarctic seawater.

The species were grown under iron limitation in the IfM-GEOMAR culture collection at 3°C. Special care was taken to prevent contamination with iron. Every procedure was done under trace metal clean conditions in a laminar flow bench. All materials coming into contact with the cultures and/or the medium were HCl rinsed before use. Sterile filtered Antarctic seawater enriched with macronutrients, vitamins, and EDTA buffered trace metals (except for iron), all in f/2 concentrations, was used as culture medium. The light climate was 30 μmol photons $m^{-2}\,s^{-1}$ provided by cool fluorescence tubes (OSRAM FLUORA L18 W/77 and BIOLUX 18 W/965) at a 16 h: 8 h light : dark cycle.

Sub samples of the same start cultures were transferred to the eight different treatments with three replicates each for every species and treatment (Table 1). The culture media for all experimental treatments was prepared as described above except for iron and silicate concentrations. Handling during the experiment was again done under trace metal clean conditions as described above. In the four low iron treatments no iron was added to the culture media, in the four high iron treatments 100 nM Fe were added. In these treatments free iron concentration were 1.55 nM Fe' (all inorganic Fe species) estimated after Timmermans et al. (2001).

The iron, silicate, and light conditions of the different treatments are shown in Table 1. The high silicate treatments were grown in 200 μM Si, which is the concentration commonly recommended in f/2 media for diatoms. The 10 times lower Si concentrations in the low Si treatments (20 μM Si) resulted in a NO_3^- : $Si(OH)_4$ ratio of 44, which is close to the ratio that can be found in low Si regions of the Southern Ocean, where Si concentrations are depleted to <1 μM (Brzezinski et al., 2005; Coale et al., 2004; Franck et al., 2000; Sigmon et al., 2002). The light : dark cycle was kept at 16 : 8 h for all treatments. All cultures were grown in 250 ml polycarbonate bottles. Before use the bottles were HCl cleaned three times for at least 48 h followed by triple rinsing with Milli-Q water.

Because of the extremely different growth behavior, sampling times and experiment periods were different between the species and partly between the treatments as well. Sampling times for cell counts, Fv/Fm, and Chl measurements are listed in Table 2.

In all high light treatments (C, D, G, and H see Table 1) of *Actinocyclus* sp. no growth was detectable based on cell counts and Fv/Fm until day 46 so the experiment was stopped thereafter. In the low light treatments (A, B, E, and F) we followed the experiment until day 77, but only treatment E showed a significant increase in cell numbers and Chl concentrations after day 46. Therefore Fv/Fm and cell counts of the treatments A, B, and F are also only shown until day 46 (Figs. 1 and 2). Chl per cell was determined at day 46 for *Actinocyclus* sp., day 21 for *Chaetoceros dichaeta*, and day 27 for *C. debilis* except for treatment F and H. Here Chl per cell was estimated at

day 9 as Chl concentrations decreased thereafter.

Samples for chlorophyll measurements were filtered on GF/F filters (Whatman) and immediately stored at –20°C until analysis. The frozen filters were put in polypropylene vials and 11 ml 90 % acetone and glass beads (2 mm and 4 mm) were added. Thereafter the closed vials were put in a cell mill for at least 5 min until the filters were completely homogenized. The vials were then centrifuged at –5°C (10 min at 5000 rpm). The extract was carefully taken by a pipette and filled in 5 cm glass cuvettes. Extinction was measured photometrically based on Jeffrey and Humphrey (1975).

The photosynthetic efficiency (Fv/Fm) was measured using a PhytoPAM (Walz, Germany) based on Kolbowski and Schreiber (1995). Samples were dark adapted for 10 minutes and kept on ice directly before measurement.

For determination of cell numbers 2 ml samples were fixed with 40 μl Lugol's Solution (iodine – potassium iodide solution 1%, MERCK) and stored at 3°C in the dark until analysis. Cells counts were performed using light microscopy (Utermöhl and Axiovert 100) at different magnifications according to the size of the organisms. In each sample at least 500 cells were counted. In the *Actinocyclus* cultures, the whole sample volume was counted because of the very low cell numbers.

Fixation with Lugol's Solution broke cell chains after some months of storage, which was not expected by the authors. We therefore decided only to use data of chain length of samples counted within one week after fixation. Unfortunately this led to an incomplete dataset. In *C. debilis* cultures data of chain length had to be taken from different days for the same reason. Chain length was taken from day 9 (treatments A, B), day 12 (treatments F, H and E) and day 15 (treatments C, D, G). Chain length of *C. dichaeta* was determined at day 21 of the experiment for all treatments.

Length and width of the cells were measured under the light microscope during counting. Assuming a cylindrical shape of the cells, cell volume was calculated using the formula volume = $\pi \cdot (0.5 \text{ width})^2 \cdot \text{length}$.

For statistical analysis Students t-test was used. Differences found are reported as significant in the text if $p<0.05$.

3 Results

3.1 Morphological changes

Iron and silicate both had an effect on cell morphology in both *Chaetoceros* species. While cells grown under iron replete conditions had a healthier appearance, iron limitation led to a visible loss in cellular chlorophyll concentrations in *C. dichaeta* and *C. debilis* (Figs. 1 and 2). Under silicate limitation a distinct elongation of the cells was observed (Figs. 1 and 2, treatment B, D, F, H). Iron and silicate co-limitation resulted in frustule malformation in *C. dichaeta* (Fig. 2, treatment B and D). No visible effect of light intensity on cell morphology could be established for both *Chaetoceros* species. *Actinocyclus* showed no clear differences in cell morphology in the different treatments. The variance in cell size and pigmentation shown in Fig. 3 was found in all treatments. However, in treatment F the cells showed to have a higher cellular chlorophyll content. All morphological changes described here were found in at least 60% of all cells; in most cases all cells were affected.

3.2 Culture development

Under typical SO conditions with low iron concentrations and a low light environment (treatment A and B) the three diatom species *Actinocyclus* sp., *Chaetoceros dichaeta*, and *C. debilis* were able to grow moderately except for *Actinocyclus* in treatment A (Figs. 4a and b). High silicate concentrations (treatment A) enhanced maximum cell numbers 14.9 times in *C. debilis* and 5.5 times in *C. dichaeta*, while *Actinocyclus* surprisingly showed no significant increase in cell numbers in treatment A. For all cultures Fv/Fm values were between 0.23 and 0.3 at the beginning of the experiment (Fig. 5) indicating iron limitation (Greene et al., 1992). Unlike *Actinocyclus*, both *Chaetoceros* species showed increasing Fv/Fm values within the first days of the experiment to a maximum of 0.4 (A) and 0.45 (B). In the low silicate cultures (B) the values decreased more rapidly and were slightly lower than those of the high silicate cultures (A) after

day 10.

Low iron and high light conditions (treatment C and D) mimic natural SO conditions under shallow mixing. This increase in light intensity compared to the treatments A and B did not increase growth of all species tested. *Actinocyclus* cultures did not grow at all under these high light conditions. Both *Chaetoceros* species showed almost no difference between the high and low light conditions under low silicate concentrations (B compared to D). In *C. dichaeta* we observed a significantly lower increase in cell numbers under high silicate and high light conditions (C compared to A). In *C. debilis* the increase in cell numbers was initially higher in the high light treatment C compared to the low light treatment A. However, the culture grew longer under low light conditions, reaching about three times higher maximum cell numbers. Fv/Fm values for *Actinocyclus* and *C. debilis* were slightly lower compared to the low light treatments A and B.

Under high iron and low light conditions (treatment E and F) growth of all cultures was higher in the high silicate treatment E compared to all low iron treatments A–D. In the low silicate treatment (F) only the growth of *Actinocyclus* was higher compared to the equivalent low iron treatments, while both *Chaetoceros* species showed similar or even lower growth. The increase in cell numbers under high silicate concentrations in treatment E was more than 10 times higher in *C. dichaeta* and almost 40 times higher in *C. debilis* compared to treatment F. Interestingly, besides absolute growth, silicate also seems to influence the growth behavior. Under low silicate concentrations (F) *Actinocyclus* had higher cell numbers in the beginning and was not overtaken by treatment E until day 42. *C. debilis* seems to have a longer lag phase under high silicate conditions as well. For *C. dichaeta* no cell counts in the beginning of the experiment were available. Fv/Fm values in both treatments were higher for all cultures compared to all low iron treatments. Maximum Fv/Fm values were highest in *C. debilis* cultures in both treatments reaching 0.69 (E) and 0.63 (F). However, in this species the Fv/Fm values decreased rapidly in treatment F after day 9.

Under high iron and high light conditions (G and H) no growth was detectable in

the *Actinocyclus* cultures and *C. dichaeta* cultures. Only the diatom *C. debilis* was able to grow and its increased in cell numbers was 20 times higher under high silicate concentrations in treatment G. Compared to the low light treatments E and F there was no significant difference in the first 27 days. However, as *C. debilis* continued growing until day 47 in treatment E, maximum cell numbers in treatment G were significantly lower. This observation is comparable to the lower maximum cell numbers in the low iron treatments C compared to A. The Fv/Fm values for all cultures were significantly lower compared to the same iron and silicate conditions under lower light (E and F). In the *Actinocyclus* cultures Fv/Fm values directly decreased below 0.2 in all treatments. While *C. debilis* showed highest maximum Fv/Fm values in treatment G (0.59) almost no change compared to the start value was observed in the low silicate treatment H. Here *C. dichaeta* cultures showed highest maximum values with 0.47.

3.3 Cellular chlorophyll and cell volume

In cultures of *Actinocyclus* sp. cellular chlorophyll concentrations were 0.08 ± 0.01 ng cell^{-1} (Fig. 6) and showed no significant difference between the treatments except for treatment F (0.16 ng cell^{-1}). In both *Chaetoceros* species cellular chlorophyll concentrations increased under low light conditions, low silicate, and under high iron concentrations. Like in *Actinocyclus*, the combination of these three factors in treatment F resulted in highest cellular chlorophyll concentrations in both *Chaetoceros* species (4.9 pg cell^{-1} in *C. dichaeta* and 0.45 pg cell^{-1} in *C. debilis*). The high cellular chlorophyll concentrations of *Actinocyclus* and *C. debilis* are clearly visible in the microscopic pictures (Figs. 1, 3).

The effect of nutrient limitation on cell size was again species specific (Fig. 7). In cultures of *Actinocyclus* cell volume was between 96 297 and 152 440 μm^3 and showed no significant changes between the eight different treatments. However, this species only grew in the three treatments B, E, and F and here cell volume was slightly lower compared to the others. In both *Chaetoceros* species cells grown under iron limitation tended to be smaller compared to the same light and silicate conditions under high

iron concentrations respectively (compare treatments A and E; B and F; C and G; D and H). However, the effect of iron on cell volume was minor and often not significant compared to the effect of silicate. In *C. dichaeta*, silicate limitation led to a significant increase in cell volume of up to 4.7 times (treatment C and D). In *C. debilis* cultures, cells grown under silicate limitation again showed a significantly higher increase in cell volume of almost three times. In both species, this increase in cell volume under low silicate conditions was caused by the elongation of cells (Figs. 1 and 2).

As both cellular chlorophyll concentrations and cell volume were affected by iron, silicate, and light, we determined chlorophyll concentrations per cell volume to be able to better compare the treatments. In *Actinocyclus* mean concentrations of chlorophyll per cell volume were 0.0006 pg μm^{-3} and showed no significant difference except for treatment F (Fig. 8). Here the values were three times higher (0.0018 pg μm^{-3}). In both *Chaetoceros* species mean chlorophyll per cell volume tended to be higher in the high iron treatments with mean values of 0.012 pg μm^{-3} in *C. dichaeta* and 0.018 pg μm^{-3} in *C. debilis*. As silicate limitation increased cell volume stronger than cellular chlorophyll concentrations, chlorophyll per cell volume was lower in the low Si treatments except for treatment F in *C. dichaeta*. High light intensities also lowered the concentrations of chlorophyll per cell volume. This effect was strongest in *C. dichaeta* under low iron concentrations.

3.4 Chain length

Chaetoceros dichaeta and *C. debilis* are both chain forming diatoms. The chain length of both species was influenced by iron, light, and silicate (Figs. 9a, b). In the low iron treatments of *C. dichaeta* cultures 90% of all cells were single cells or in 2–3 cell chains (Fig. 9a). The chain length in the iron replete treatments seemed to be influenced more by light and silicate availability than in *C. debilis* cultures. No significant changes compared to the low iron treatments were found in treatments G and H. Longest chains were found in the high iron, low light treatment E where 50 % of all cells were in chains of 3–5 (E) cells. In treatment F there also was a small tendency towards longer chains.

In all *C. debilis* cultures grown under iron limitation, 90% of all cells were single cells or in two cell chains (Fig. 9b). High iron concentrations resulted in an increase in chain length except for the high light, low silicate treatment (H). The increase in chain length was highest in the two high iron, high silicate treatments G and E. Here 50% of all cells were in chains of 1–4 (E) and 2–5 (G) cells. Additionally, in treatment G up to 16 cells per chain were occasionally observed (<5%, data not shown). In the high iron, low light, low silicate treatment (F) the increase in chain length was slightly higher compared to the low iron treatments.

4 Discussion

The Southern Ocean is the largest HNLC region of the worlds' oceans where various factors suppress growth of primary producers despite the generally high nitrate concentrations. The low iron concentrations in the SO are known to limit algal growth in general while diatoms are additionally limited by low silicate concentrations north of the PFZ. The wind mixed layer depth in the SO is generally high and can reach up to about 100 m after storm events (de Baar et al., 2005). Because of these deep mixing events the phytoplankton cells are often exposed to very low light intensities, which are thought to additionally limit the photosynthetic activity and thus growth.

In this study we examined the effect of co-limitation of the three main parameters that may limit diatom growth in the SO: iron, silicate, and light in laboratory experiments. We are aware that laboratory experiments can only try to imitate nature and never create a truly natural environment. However, while focusing on certain key variables under controlled laboratory conditions, information about some adaptation strategies will be obtained.

The nutrient concentrations in culture media are usually much higher compared to natural conditions. This is necessary to reach sufficient biomass in a relatively small volume so that there is enough material for analysis. Nutrient concentrations that would be considered high in the field, such as the 20 μM silicate, were suitable for our low Si

treatments due to the much higher biomass and showed to reduce algal growth in our experiments.

4.1 The effect of nutrient limitation on morphology

The SO is known to be a major sink of biogenic opal in the worlds' ocean and most of this opal consists of diatom frustules that grow in the euphotic zone and sink to the seafloor in aggregates or fecal pellets. Frustules found in SO sediments are commonly used to reconstruct past variations in sea surface temperature (Crosta et al., 2005; Kunz-Pirrung et al., 2002), sea ice cover (Armand et al., 2005; Gersonde and Zielinski, 2000), drifting of tropical/subtropical species to higher latitudes (Romero et al., 2005), and to trace pathways of Antarctic Bottom Water (Zielinski and Gersonde, 1997). Thereby often the identification down to species level is essential as some species can be used as indicators for the above mentioned parameters. Nutrient limitation that may affect frustule morphology as described in this study can therefore cause difficulties in species identification and lead to misinterpretation.

The genus *Chaetoceros* is one of the most abundant diatom genera in the ocean and its distribution covers most environments from coastal temperate to polar regions. The genus is composed of about 180 species and many of them are found in the Southern Ocean. Usually *Chaetoceros* species are relatively slightly silicified compared to other diatoms, which makes them more sensitive to remineralization and leads to poorly preserved siliceous remains in sediments of the open SO. *Chaetoceros* is known to prefer nutrient rich conditions and in the SO this genera is mainly found in near shore sediments (Armand et al., 2005; Zielinski and Gersonde, 1997). In the East Antarctic Margin *Chaetoceros* species dominate the phytoplankton community and partly pure *Chaetoceros* ooze can be found in the sediments (Stickley et al., 2005). In these regions, *Chaetoceros* frustules in the sediment are used as an indicator for seasonal changes and spring sea ice melting events (Stickley et al., 2005), which are known to increase nutrient concentrations. Our data show that besides the known effects of remineralization and destruction due to grazing, frustule morphology is also affected

by nutrient availability. Under Si and Fe co-limitation the frustules of *C. dichaeta* were clearly malformed and fragile while *C. debilis* and *Actinocyclus* did not show such morphological changes. This observation could be another reason for the poorly preserved *Chaetoceros* frustule in the sediments of the open SO and it is possible that *Chaetoceros* abundances in the euphotic zone are thus underestimated. To our knowledge no such malformation of SO diatom frustules in the field is described in the literature and we can not exclude that part of these morphological changes may be artifacts due to culturing. However, it is very likely that, if they are abundant in the field, malformed frustules would not be identified correctly if found in water samples or sediments. Even though it will not be possible to exactly count cell numbers if the frustules are malformed and poorly preserved, the recognition of those could be a hint for iron and silicate co-limitation in the euphotic zone. Our data could therefore provide a helpful contribution to visually identify nutrient limitation in *C. dichaeta* and possible other SO diatoms.

The observation that cells of both *Chaetoceros* species were elongated under silicate limitation are similar to those reported by Harrison et al. (1977). This suggests that low silicate concentrations not only influence the build up of new frustule material but also the mechanism of cell division itself. The cell cycle is classically divided in four phases the G1, S, G2 and M. While DNA is replicated during the S phase and mitosis and cell division take place in the M phase, G1 and G2 refer to "gaps" in between those processes. During these "gaps" most of the cell growth takes place (see review in Ragueneau et al., 2000). Silicon uptake and the formation of new frustules by diatoms are non-continuous processes that are confined to the G2 phase (Brzezinski, 1992; Brzezinski et al., 1990). It is described in the literature that nutrient limitation in general and resulting low growth rates leads to elongated G2+M phases and thus increased total silicate uptake (Claquin et al., 2002). However, cells grown under Si limitation may not be able to reach a certain intracellular silicate concentration. They may remain in the G2 phase and therefore do not enter the M phase and do not divide. We can only speculate what causes the extreme elongation of the cells under Si limitation (Figs. 1 and 2), but they may have some kind of regulatory process that stops them from di-

viding until they have collected a minimum amount of silicate as the new frustules may otherwise be too fragile. However, we observed that the cells continued building up plasma and that the girdle band continued growing, which resulted in the elongated shape. Similar morphological changes are described for diatoms under silicate limitation in the field and in laboratory experiments (Harrison et al., 1977; Paasche and Østergren, 1980) and are also explained by continued cell growth while cell division is blocked.

Besides the effect on growth and cell morphology, iron and silicate also influenced chain length in both *Chaetoceros* species (Fig. 9). Both species had the highest number of cells per chain under high iron and high silicate conditions. In *C. debilis* this was independent of light intensity while in *C. dichaeta* chain length was only longer in the low light treatment. The formation of chains is an important and effective way to prevent grazing (Fryxell and Miller, 1978; Pahlow et al., 1997). Besides the species specific effects of iron, light, and silicate on growth, such differences in grazing protection will have additional impacts on species composition under changing nutrient concentrations in the SO. Based on these findings we propose that under favorable nutrient conditions the cosmopolitan species *C. debilis* can benefit from long cell chains as grazing protection independent of the season while *C. dichaeta* would be at a disadvantage at higher light intensities during periods of shallow mixing.

4.2 The effect of light intensity

Light limitation is thought to be one major reason for low phytoplankton biomass and drawdown of nutrients in the euphotic zone of the SO. Mitchell et al. (1991) modeled that under the deep mixing conditions given in the SO, only ~10% of the available nutrients could be utilized due to light limitation. In accordance with this model, a negative correlation between WML depth and chlorophyll concentrations (mg m^{-3}) was observed in in situ iron fertilization experiments (de Baar et al., 2005). However, when integrated to mixed layer depth, chlorophyll concentrations during EIFEX were the highest compared to all other in situ iron fertilization experiments despite the very deep

mixing (Peeken et al., unpublished data).

The importance of iron for photosynthesis stems from high concentrations in the photosystem I and II and the cytochrome b_6f complex (Raven, 1990). Under low light intensities the production of light-harvesting pigments is enhanced and thus the cellular iron requirements increase (Strzepek and Price, 2000; Sunda and Huntsman, 1997). In regions like the SO where iron is limiting, low light intensities are therefore likely to co-limit phytoplankton growth. However, it has been described recently that the oceanic diatom species *Thalassiosira oceanica* had a much lower concentration of the iron rich parts of the photosynthetic apparatus, photosystem I and cytochrome b_6f complex, compared to the coastal species *T. weissflogii* (Strzepek and Harrison, 2004). This leads to a significant decrease in cellular iron demand while growth and photosynthetic efficiency stayed at a high level, comparable to those of the coastal species. Whereas the exact physiological mechanisms remain unknown so far, this apparent paradox is explained by a higher effective absorption cross-section and turnover rate of photosystem I in the open ocean species, possible in adaptation to the low natural iron concentration (Strzepek and Harrison, 2004). Similar adaptation strategies could enable SO diatoms to sustain high growth under iron and light conditions that would limit other species.

In this study we could not find a general limiting effect of low light intensity. *Actinocyclus* sp. and *C. dichaeta* were clearly not light limited grown under 30 μmol photons $m^{-2}\,s^{-1}$. This equals about the light intensity in 16 to 42 m depth, depending on surface radiation in the open SO during EIFEX (R. Röttgers, personal communication). However, in the field phytoplankton cells are never exposed to constant light intensities but undergo permanent changes in the light climate due to mixing and changes in weather conditions. Assuming surface irradiances between 100 and 500 μmol photons $m^{-2}\,s^{-1}$, phytoplankton cells would be exposed to mean light intensities of 30 μmol photons $m^{-2}\,s^{-1}$ when constantly mixed between the surface and 44 m and down to calculative more than 200 m respectively. Although this assumption is very theoretical it suggests that mixing depths of about 100 m, as commonly observed in the SO, may on average not result in a limiting light climate.

In our experiments, an increase by a factor of three to 90 μmol photons m^{-2} s^{-1} (mean light intensity in 1–28 m depth) at the same light : dark cycle suppressed growth and photosynthetic efficiency of *Actinocyclus* under low and high iron concentrations and of *C. dichaeta* under high iron concentrations. In contrast to our findings, laboratory experiments with single species and deck incubations with natural phytoplankton assemblages suggest an iron and light co-limitation of the SO phytoplankton. Although these experiments are difficult to compare as some laboratory experiments were not performed with SO phytoplankton species (Strzepek and Harrison, 2004; Strzepek and Price, 2000; Sunda and Huntsman, 1997) and light intensities differ from 20 to about 900 μmol photons m^{-2} s^{-1} and from light : dark cycles of 12 : 12 to 24 : 0 h (de Baar et al., 1990; Martin et al., 1990; Sunda and Huntsman, 1997; Timmermans et al., 2001), it can be summarized that smaller species are reported to be less affected by iron and light co-limitation compared to larger ones. Timmermans et al. (2001) for example report that *C. dichaeta* was only able to grow at a light : dark cycle of 20 : 4 h at 80 μmol photons m^{-2} s^{-1} while no growth was detected under the same light intensity at a light : dark cycle of 12 : 12 h. They conclude that *C. dichaeta* is iron and light co-limited under short day conditions. However, in these experiments the absolute amount of photons during one light period was 3.46 mol photons m^{-2}, which is exactly twice as much as in our low light experiments (1.73 mol photons m^{-2}). This shows that the duration of irradiance is more important than the light intensity itself. The light : dark cycle in culture experiments simulates the time of year and therefore gives no information about possible reactions to changing WML depth.

Adaptation to low light in the SO was observed during the in situ iron fertilization experiment EIFEX. Although it is generally assumed that no net growth is possible below the 1% light depth, relatively high primary production of 3.4 mg C m^{-3} d^{-1} was observed at depth with 0.1% of the surface light intensity (Peeken et al., unpublished data). The phytoplankton community of the SO is therefore able to maintain positive growth at extremely low light intensities. Similar adaptation strategies are also known for ice algae and benthic diatoms, which usually only get less than 0.1% of the surface

light intensities (Admiraal, 1977; Thomas and Dieckmann, 2002). However, to our knowledge no such adaptation strategies are reported for pelagic diatoms in the SO.

Grown under high light intensity the three species tested here showed very different responses. While *Actinocyclus* was not able to grow in any of the high light treatments, *C. debilis* seems to be able to deal with higher light intensities as the increase in cell numbers was not significantly higher in most of the low light treatments. *C. debilis* is not endemic in the SO but more or less globally distributed (Anderson et al., 2004). This means that this species has to be adapted to a variety of very different light and nutrient environments. Besides, *C. debilis* may be more susceptible to grazing than larger species. Being able to sustain high growth rates under varying environmental conditions can therefore be essential to survive. Under shallow mixing conditions such "generalists" as *C. debilis* are likely to have an advantage over low light adapted species. Surprisingly *C. dichaeta* was able to grow under high light and low iron, while no growth was detected under high light and high iron. *C. dichaeta*, as a SO species, is subjected to the deep mixing of the SO and the resulting low light conditions. When iron becomes available this species may start to increase its photosynthetic efficiency to use the low light as effective as possible. It may then not be able to deal with a surplus of light energy.

Several studies show that species specific differences in the level of iron and light co-limitation exist (Sunda and Huntsman, 1997; Timmermans et al., 2001). Timmermans et al. (2001) conclude from their findings that mainly larger diatoms are iron and light co-limited and that low iron and low light conditions in the SO will favor the growth of small diatoms. The reason why those can not build up high biomasses is assumed due to higher grazing pressure. The importance of grazing for the size distribution of the phytoplankton community will be discussed below. In contrast to Timmermans conclusion, our findings show that the large diatoms species tested here (*Actinocyclus* sp. and *C. dichaeta*) are not light limited but that higher light intensities have a negative effect on growth. A shallower mixing and the resulting higher irradiance would therefore not favor the growth of these larger diatom species. The observation that diatom

blooms in the SO are dominated by large diatoms highlights the importance of grazing to suppress the biomass of smaller diatoms.

In conclusion we assume that the importance of light limitation in the SO is overestimated. On the other hand the importance of grazing to control the biomass of smaller diatoms and to allow larger species to bloom may have been underestimated. The species specific differences in the interaction of iron and light found here and in the literature may help to complete our understanding of the development of diatoms blooms under different environmental conditions in the SO.

4.3 The effect of Fe and Si limitation on diatom growth

In situ iron fertilization in the SO showed that community growth was more enhanced by iron addition in high silicate waters compared to low silicate waters (Coale et al., 2003, 2004; Leblanc et al., 2005). Similar to these findings from the field, growth of all three species tested in this study was clearly co-limited by iron and silicate, as highest cell numbers were reached in the high iron, high silicate, low light treatment.

Nutrient requirements are generally assumed to be linked to cell size as uptake rates are dependent on the surface to volume ratios (Chisholm, 1992; Morel et al., 1991). Thus smaller species are less affected by nutrient limitation compared to larger species. In this context Timmermans et al. (2001) describe that growth of the small Antarctic diatom *C. brevis* was not limited by low iron concentrations while the larger *C. dichaeta* was. In contrast to that a positive effect of iron on growth of the small diatom species *Fragilariopsis cylindrus*, *Cylindrotheca closterium*, *Chaetoceros* sp., and one unidentified pennate diatom during the in situ iron fertilization experiment EIFEX was described by Hoffmann et al. (2006). Sedwick et al. (2002) suggested that larger diatom species might be more silicate limited and that these species therefore bloom in high silicate waters when iron becomes available. In agreement to that enhanced growth of small pennate diatoms with iron addition in high and low silicate waters is described by Hutchins et al. (2001). They assume that these small, lightly silicified species are highly adapted to low Si growth conditions. However, our results demon-

strate that there are small species that do not react in this way. The negative effect of silicate limitation on growth was highest in *C. debilis*, the smallest species, and lowest in *Actinocyclus*, the largest species tested. Larger, strongly silicified species have a higher amount of silicate per cell and in absolute numbers more silicate is needed to build up new frustules. However, relative to cell volume the amount of silicate may be even higher in small species. Especially in combination with high growth rates, such small species may be limited earlier by low silicate concentrations than slow growing larger species. Further, the extent of Fe and Si co-limitation on growth of the three diatom species was again higher in the smallest species. Under low light conditions maximum cell numbers were 36 times (*C. debilis*), 7 times (*C. dichaeta*), and 3 times (*Actinocyclus*) lower under Fe and Si co-limitation compared to Fe and Si replete conditions. Thus our data suggest that the extent of iron and silicate co-limitation is not only dependent on cell size. The differences between the species tested here and others reported in the literature suggest that the influence of nutrient co-limitation in the SO is even more complex than hitherto assumed.

Possible explanations for these observations are differences in the physiological adaptations to nutrient limitation, such as the number and activity of membrane transport proteins, that might compensate the effect of cell size. It is generally accepted that iron limitation decreases the maximum specific uptake rate (V_{max}) for silicic acid in marine diatoms, while absolute values of V_{max} differ between species (De La Rocha et al., 2000; Franck et al., 2003; Leynaert et al., 2004). This is explained by a decrease in the number of active silicate transporters in the cell membrane under iron stress (De La Rocha et al., 2000). Alternatively, it is suggested that, as Si uptake in marine diatoms is linked to aerobic respiration, iron limitation decreases the electron transport efficiency of the iron rich respiratory chain and thus causes a decrease in V_{max} (Franck et al., 2003). Thus iron limitation decreases the capacity for silicic acid uptake in marine diatoms. This may be of less meaning in the high silicate regions of the SO, but north of the PFZ iron limited diatoms will be even faster co-limited by the low silicate availability. In this study we could not find a positive effect of iron addition under sili-

cate limitation on growth of all three species tested. However, in *C. dichaeta* frustule malformation was only observed under iron and silicate co-limitation, while under low silicate and high iron conditions cells were elongated but frustules showed no visible malformation. This suggests a better silicate uptake under increased iron availability in this species. Brzezinski et al. (2005) hypothesize that diatom growth rates are limited by iron while biogenic silica production rates and cellular silicon content may be controlled by a combined influence of both iron and silicate. Our data show that iron and silicate both have a direct influence on diatom growth. Under silicate limitation both *Chaetoceros* species tested seemed to have problems reaching their intracellular silicate concentration needed for cell division. This is supported by the observation that cell volume is significantly higher in all low silicate treatments.

Besides the general decrease in V_{max}, the half saturation constant for silicic acid uptake (K_{Si}) is extremely different between diatom species and shows no collective trend under iron limitation (De La Rocha et al., 2000; Franck et al., 2003; Leynaert et al., 2004). This suggests that while iron may have an effect on the number of active Si transporters, their affinity for silicic acid is not Fe dependent and represents species specific properties (De La Rocha et al., 2000). Brzezinski et al. (2005) reported a decrease in K_{Si} during the in situ iron fertilization experiment SOFEX in the low Si waters of the north patch. They suggested that either iron lowers the half saturation constants for silicic acid of individual species or causes a species shift that favors diatoms with higher Si affinity. Besides the effect of the surface to volume ratio on nutrient uptake rates, species specific adaptation mechanisms such as the amount and activity of transport proteins in the cell membrane may have an important impact on iron and silicate uptake rates and therefore determine the level of iron and silicate co-limitation in SO diatoms.

4.4 Implications for the field

Artificial iron fertilization is performed with the aim to decrease atmospheric CO_2 concentrations by increasing the carbon export to the deep sea in HNLC regions. It is

therefore important to investigate the effect of iron fertilization on the community structure and carbon export in high and low Si waters of the SO.

If persistent iron fertilization in low silicate waters would shift the phytoplankton community towards diatom species with lower K_{Si} and/or those who are able to decrease K_{Si} with increasing iron availability, these species would be able to increase biomass and therefore would rapidly deplete the Si concentrations. This is consistent with findings of Coale et al. (2004) who report that even though diatoms only accounted for less than 50% of the total biomass under low Si conditions, this phytoplankton group showed the highest increase relative to initial values after iron fertilization in the low silicate SOFEX north patch. After a diatom bloom biogenic material is known to be effectively exported to the deep sea via aggregation. Therefore it can be suggested that the system would remain Si depleted. The resulting nutrient environment would make it impossible for diatoms to bloom until the next upwelling event supplies new Si. Other phytoplankton classes as prymnesiophytes, pelagophytes, and dinoflagellates would have the advantage and it is possible that they would dominate the phytoplankton community permanently. Diatoms are known to play a major role in carbon export to the deep sea, as the heavy silicate frustules have high sinking rates (Waite and Nodder, 2001). Other phytoplankton groups have less effective protection mechanisms as frustules and formation of cell chains, and are therefore exposed to higher grazing pressure compared to diatoms. Thus biomass in a non diatom dominated system would be mainly exported as fecal pellets and remineralization and recycling production would be more effective (Dubischar and Bathmann, 2002). It can be speculated that persistent iron fertilization in low silicate waters and a possibly resulting shift in species composition towards a lower diatom contribution would therefore result in a system with less efficient carbon export.

Our results suggest that iron fertilization under high silicate conditions supports growth of large and small diatoms. The general observation that large diatoms bloom in the SO when iron becomes available (see review in de Baar et al., 2005; Hoffmann et al., 2006) shows that other factors such as grazing determine species succession

in an effective way. As mentioned above small grazers are very efficient in controlling the biomass of the small phytoplankton community. Large diatom species are known to be better protected against these grazers because of their size, the silica frustules, and the formation of cell chains. In addition Dubischar and Bathmann (1997) reported that large copepod grazers, that are able to ingest large diatom species and cell chains, had a more patchy distribution and ingestion rates were to low to control the biomass of these species under favorable nutrient conditions. Only the very randomly distributed salps had ingestion rates high enough to potentially suppress such a bloom. Of the species tested in this study, *C. debilis* seems to be able to adapt best to changing environmental conditions and maintain favorable growth rates. These findings in our laboratory experiments are supported by field observations from the subarctic Pacific Ocean. It has been shown that *C. debilis* is able to exceed the growth of other species in the field and become the dominant species after in situ iron fertilization (Tsuda et al., 2003). The growth of *C. dichaeta* and especially *Actinocyclus* were much more affected by the availability of iron, light, and silicate. However, growth of all species showed to be co-limited by iron and silicate. If nutrient availability would be the only limiting factor of growth we would expect small "generalists" as *C. debilis* to dominate in the Southern Ocean.

In conclusion we suggest that while all diatom size classes may be able to increase growth following iron fertilization in high Si regions of the SO, the dominance of large species might be mainly caused by effective grazing control of the small phytoplankton biomass.

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Table 1. Iron and silicate concentrations and light intensities of the eight treatments A-H.

Treatment	Iron	Light μmol photons m^{-2} s^{-1}	Silicate μM
A	No addition	30	200
B	No addition	30	20
C	No addition	90	200
D	No addition	90	20
E	1.55 nM Fe'	30	200
F	1.55 nM Fe'	30	20
G	1.55 nM Fe'	90	200
H	1.55 nM Fe'	90	20

Table 2. Sampling times of cell counts, Fv/Fm, and Chl measurements in the three experiments.

Day	Cell counts	Fv/Fm	Chl
	Actinocyclus sp.		
0	All treatments	All treatments	All treatments
3	All treatments	All treatments	
7	All treatments	All treatments	
11	All treatments	All treatments	
21	All treatments	All treatments	
28	All treatments	All treatments	
36	E, F, G, H	All treatments	
42	All treatments	All treatments	
46	All treatments	All treatments	All treatments
57	A, B, E, F	All treatments	
77		All treatments	A, B, E, F
	Chaetoceros dichaeta		
0	All treatments	All treatments	All treatments
3		All treatments	
7		All treatments	
10		All treatments	
13		All treatments	
16		All treatments	
18		All treatments	
21	All treatments	All treatments	All treatments
23	A, C, E	All treatments	
28	B, C, D, F, G, H	All treatments	
31		All treatments	
35		All treatments	
	Chaetoceros debilis		
0	All treatments	All treatments	All treatments
6		All treatments	
9	A, B, D, E, F, G, H	All treatments	All treatments
12	A, B, C, D, E, F, H	All treatments	
15	C, D, F, G	All treatments	All treatments
21		All treatments	All treatments
27	All treatments	All treatments	All treatments
30			All treatments
47	A, E	All treatments	All treatments

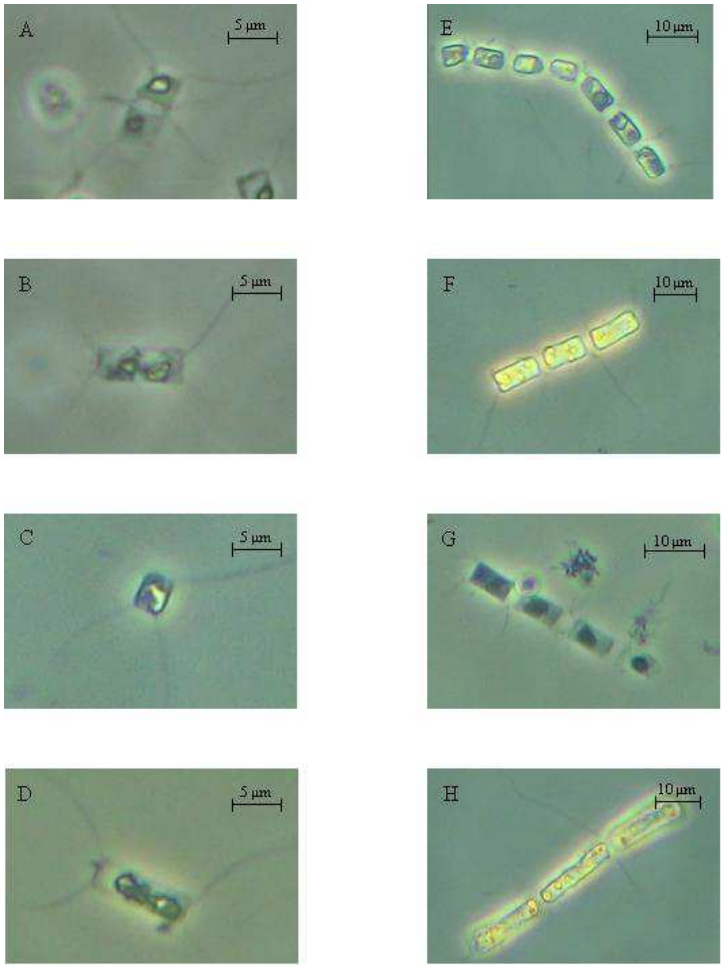

Fig. 1. Light microscopy pictures of *C. debilis* in the treatments A-H.

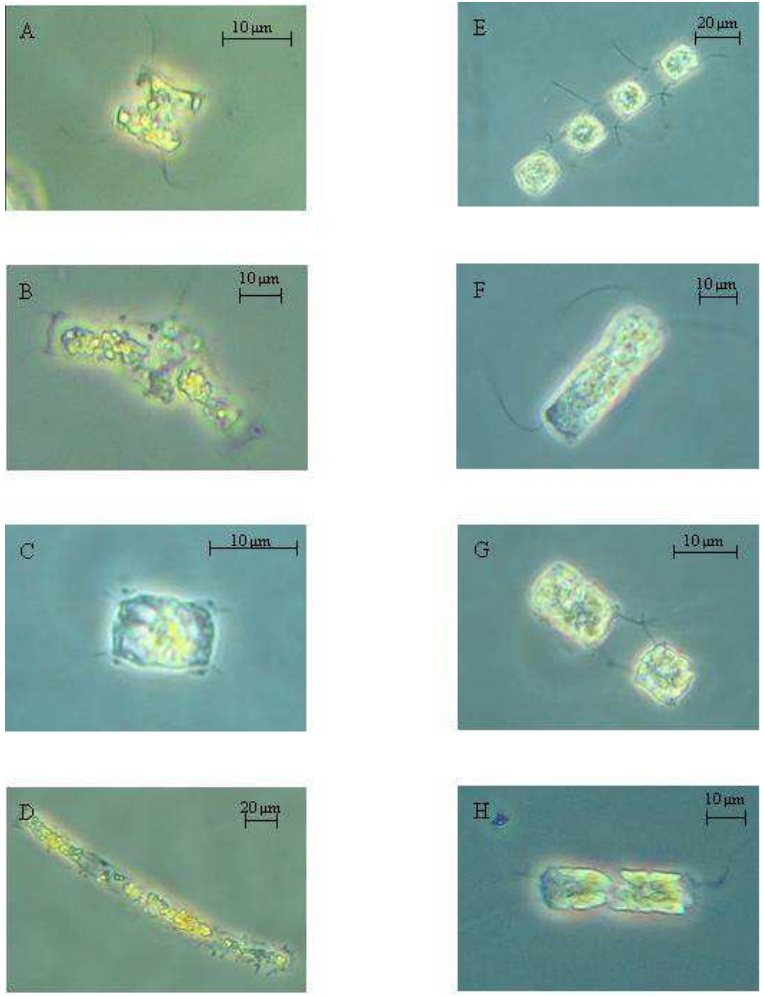

Fig. 2. Light microscopy pictures of *C. dichaeta* in the treatments A-H.

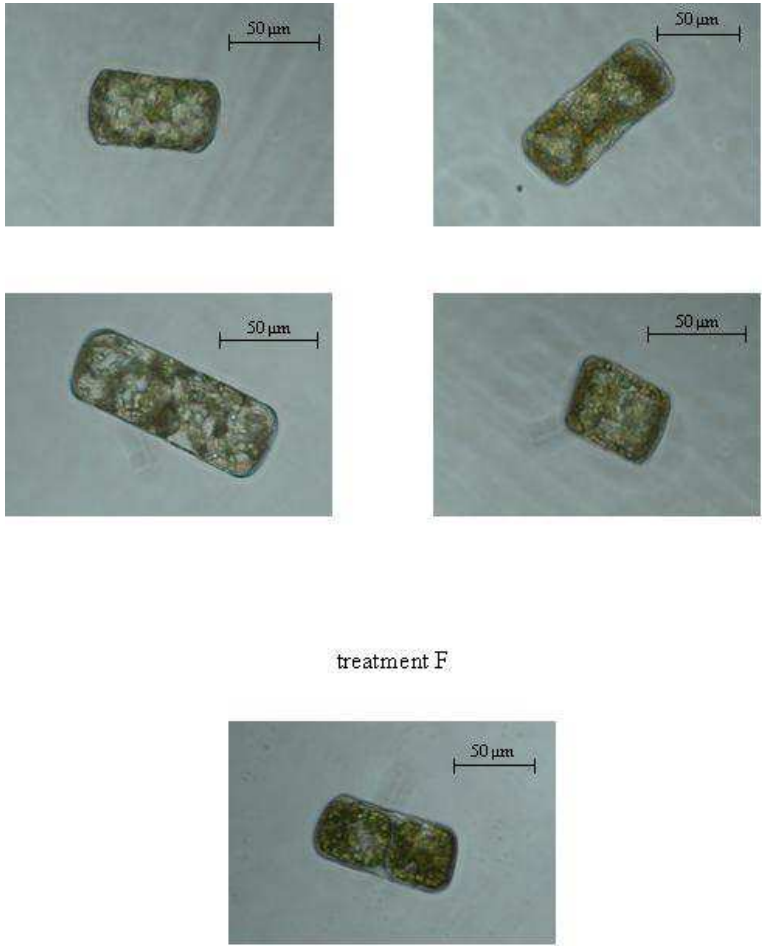

Fig. 3. Light microscopy pictures of *Actinocyclus* sp. The variance in cell size and Chl content was found in all treatments. In treatment F (picture of dividing cell) all cells had visibly higher Chl concentration per cell.

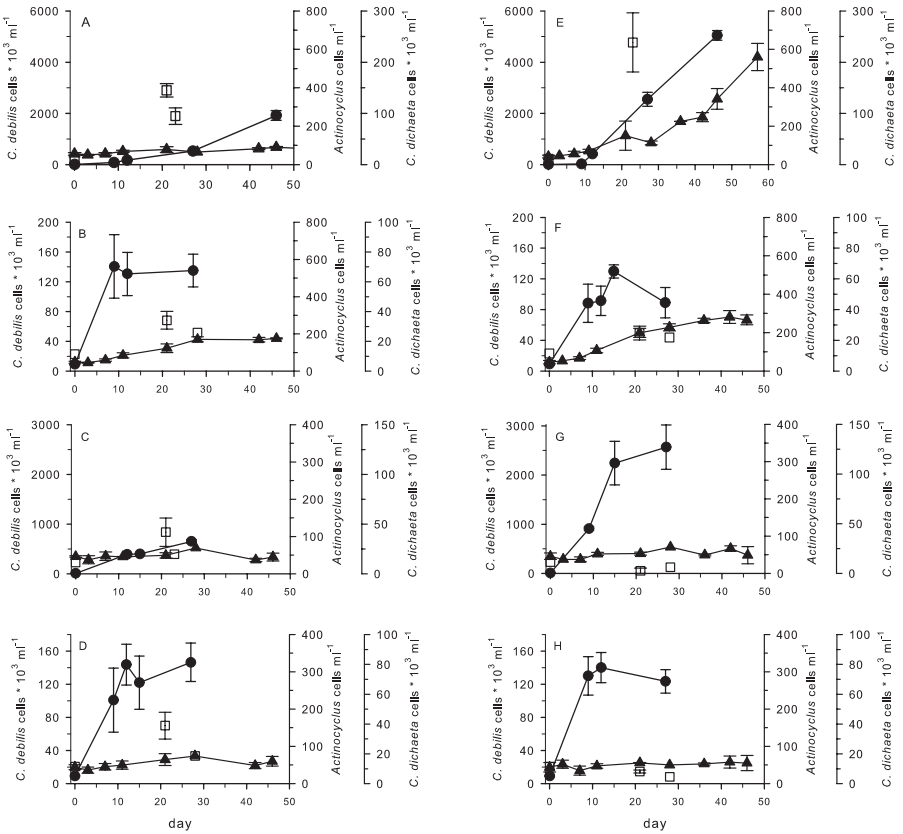

Fig. 4. Cell counts of the three species *Actinocyclus sp.* (dark triangles), *Chaetoceros dichaeta* (open squares), and *C. debilis* (dark circles) grown at the eight different treatments A-H. Note different scales for different treatments.

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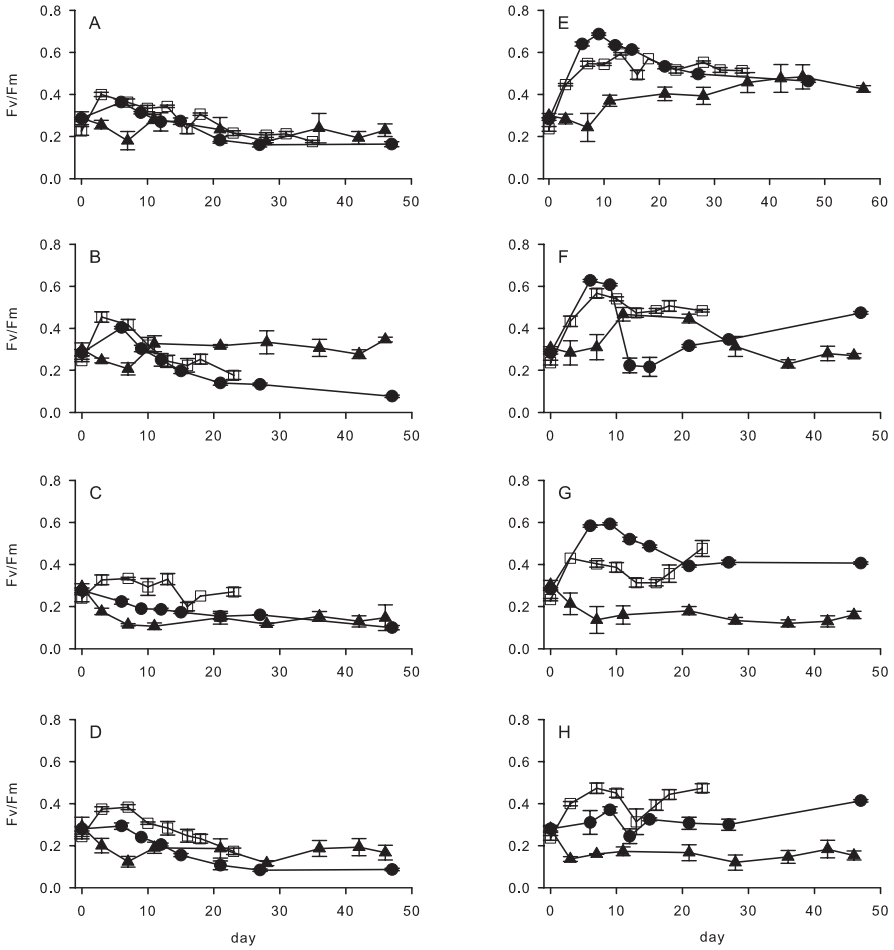

Fig. 5. Fv/Fm of the three species *Actinocyclus* sp. (dark triangles), *Chaetoceros dichaeta* (open squares), and *C. debilis* (dark circles) grown at the eight different treatments A-H.

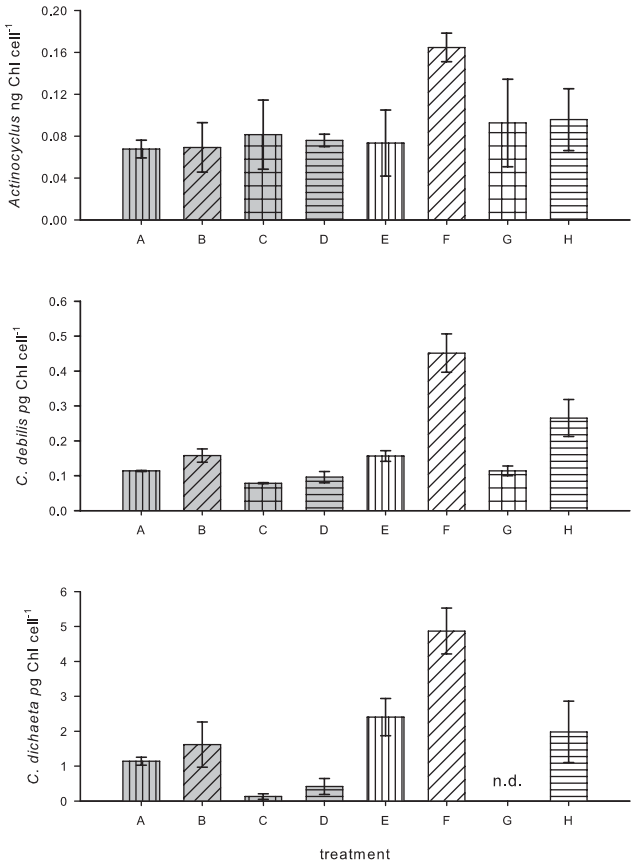

Fig. 6. Cellular chlorophyll concentrations of the three species *Actinocyclus* sp. (day 46), *Chaetoceros dichaeta* (day 21), and *C. debilis* (treatment F and H day 9, all others day 27) grown at the eight different treatments. For the *C. dichaeta* cultures chlorophyll values for treatment G are missing so no chlorophyll per cell values could be estimated.

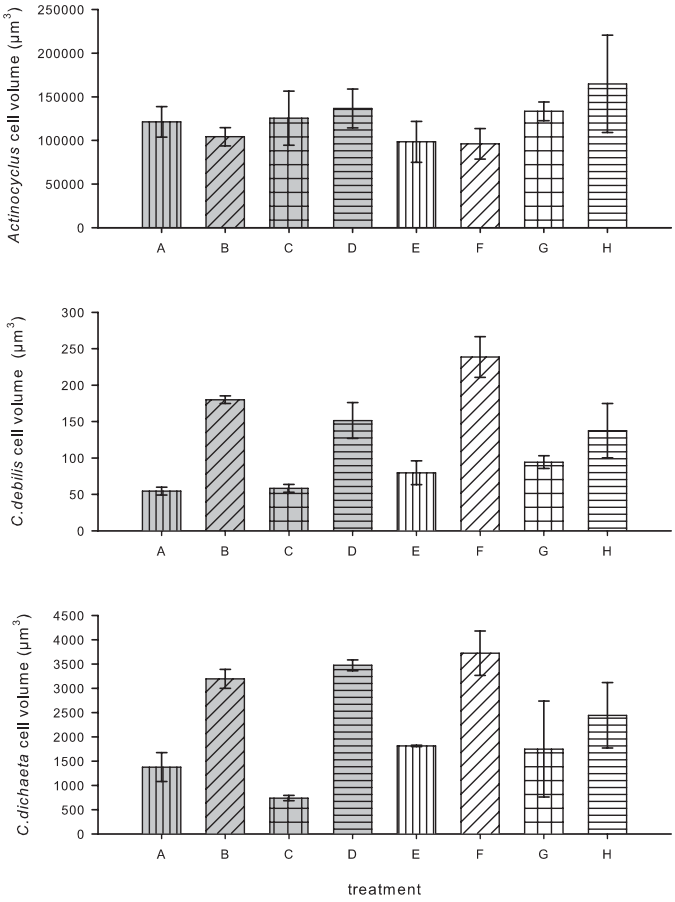

Fig. 7. Cell volume of the three species *Actinocyclus* sp. (day 46), *Chaetoceros dichaeta* (day 21), and *C. debilis* (day 27) grown at the eight different treatments.

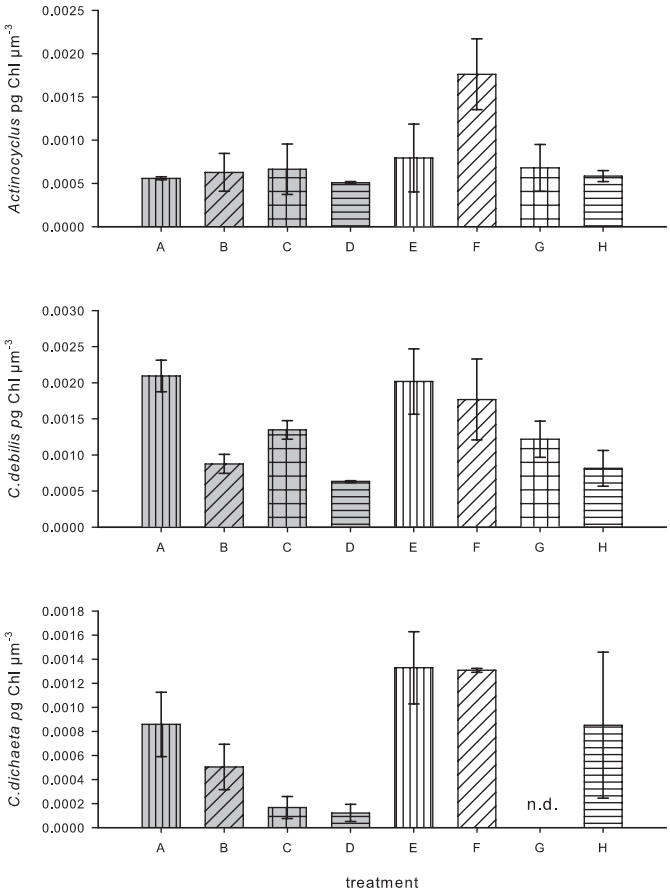

Fig. 8. Chl per cell volume in *Actinocyclus* (day 46), *Chaetoceros dichaeta* (day 21), and *C. debilis* (day 27) grown at the eight different treatments. For the *C. dichaeta* cultures chlorophyll values for treatment G are missing so no chlorophyll per volume values could be estimated.

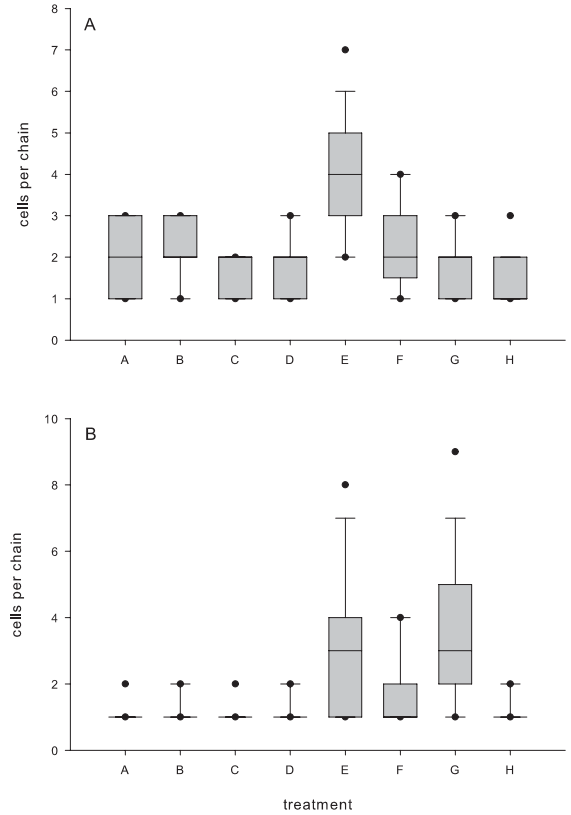

Fig. 9. Chain length of *C. dichaeta* at day 21 (A) and *C. debilis* (B) at day 9 (treatments A, B), day 12 (treatments F, H and E) and day 15 (treatments C, D, G). The boundary of the box closest to zero indicates the 25th percentile, the line within the box marks the median, and the boundary of the box farthest from zero indicates the 75th percentile. Whiskers (error bars) above and below the box indicate the 90th and 10th percentiles and dark points indicate the 95th and 5th percentile.